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A common genetic variant of *fucosyltransferase 2* correlates with serum carcinoembryonic antigen levels and affects cancer screening in patients with primary sclerosing cholangitis

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Abstract

Background: Primary sclerosing cholangitis (PSC) patients are at increased risk of biliary tract cancer, and carcinoembryonic antigen (CEA) serum levels might be used for screening.

Objective: To examine cancer screening with CEA in PSC patients and analyse how serum CEA levels are affected by genetic variants of *fucosyltransferase* (*FUT*) *2* and *3*.

Methods: In a retrospective cohort analysis we evaluated CEA levels in 226 PSC patients, including 19 with biliary malignancy, and investigated how *FUT2* and *FUT3* SNPs affected CEA levels. Receiver-operating-characteristic (ROC) analysis was performed and cut-off values were determined based on Youden's index. A control cohort contained 240 patients, including 28 with biliary malignancy.

Results: Median CEA concentration was lower in cancer-free patients (1.4 ng/mL) than in cancer patients (2.0 ng/mL, $P = 0.014$). ROC analysis revealed an area under the curve (AUC) of 0.671, the optimal cut-off was 3.2 ng/mL. The *FUT2* variant *rs601338* (G428A) correlated with CEA levels, and the effect was most prominent in a subgroup of patients genetically incapable of expressing CA19-9. The AUC improved if ROC analysis was performed separately for wild-type (AUC: 0.731) and homozygous mutant (AUC: 0.816) G428A. The influence of *FUT2* on CEA was confirmed in the control cohort.

Conclusions: CEA is interesting for biliary-malignancy screening in PSC patients, especially in patients who do not express CA19-9. This is the first study to show that the combined use of CEA measurement and *FUT* genotyping is clinically beneficial and that it might enhance the early detection of biliary malignancy in clinical practice. This approach could also be effective when screening for other common gastrointestinal malignancies.

Keywords

Primary sclerosing cholangitis, cholangiocarcinoma, gallbladder carcinoma, fucosyltransferase, carcinoembryonic antigen

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Introduction

Primary sclerosing cholangitis (PSC) is characterized by chronic inflammation of bile ducts, and it is associated with an increased risk of biliary tract cancer.[1,2] PSC patients face the risk of developing benign dominant stenoses of the major bile ducts,[3] which are challenging to distinguish from malignant strictures.[4] The lifetime risk for cholangiocarcinoma (CCA) in PSC patients is 5%–15%, with an annual incidence of 0.5%–1.0%. Moreover, these patients are at increased risk of

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developing gallbladder carcinoma (GBCA), which occurs in approximately 3%–4% of patients.[5] Consequently, the overall risk of biliary malignancy is approximately 160 times higher compared to the general population.[6]

The parameter most commonly used in screening for biliary malignancy in PSC patients is the carbohydrate antigen CA19-9. We recently showed that the use of cut-off values determined based on the genotype of *fucosyltransferase* (*FUT*) *2* and *3* improves the diagnostic value of CA19-9 in PSC patients.[7] Besides CA19-9, carcinoembryonic antigen (CEA) can be used to screen for biliary malignancies.[8] A recent genome-wide association study (GWAS) revealed an association between serum CEA levels and genes for fucosyltransferases and AB0 blood group.[9]

The enzymes FUT2 and FUT3 are capable of catalyzing the fucosylation of glycoproteins. Both enzymes are known for their importance in synthesizing CA19-9. The FUT3 enzyme catalyzes the final step of CA19-9 biosynthesis, thus its inactivation results in a loss of CA19-9 biosynthesis. The FUT2 enzyme is as well involved in CA19-9 biosynthesis. Yet in contrast, its inactivation leads to increased serum levels of CA19-9. This is due to the fact that the FUT2 enzyme modifies a CA19-9 precursor substance, which is not available for CA19-9 biosynthesis any longer after modification by FUT2. Thus in case of a loss of FUT2 enzyme activity more of this precursor substance is available for CA19-9 biosynthesis. With regard to the Lewis blood group antigen system the FUT3 and FUT2 enzyme are as well known as the Lewis and Secretor enzyme and determine the patient's Lewis blood group.[10] The *FUT2* gene, which defines the secretor type in the Lewis blood group system, was genetically determined to be inactive in approximately 20% of Caucasians,[11] and the *rs601338* variant of *FUT2* (also referred to as G428A) was reported to be the most common variant present in Europeans.[12] In a previous study, the use of the Lewis blood group system resulted in a low number of false results in comparison with a genotyping approach,[13] thus the later seems superior for determining the patients FUT2 and FUT3 enzyme activity.

In this study, we aimed to investigate the influence of common *FUT2* and *FUT3* SNPs on serum CEA levels in PSC patients and, if possible, to improve the diagnostic performance of CEA in the detection of biliary malignancy in these patients.

Patients and methods

Inclusion and exclusion criteria

Patients that were included in a previous analysis on CA19-9 were screened for inclusion in this study.[7] In addition 83 further patients were newly screened at the University Hospital of Heidelberg. Overall, patients with diagnosis of PSC that were treated either at the University Hospital Heidelberg (Germany) or Oslo University Hospital (Norway) were eligible for inclusion, regardless of the presence of biliary malignancy. For all patients, we only included data obtained prior to transplantation. Histopathological confirmation of biliary malignancy or cytology plus a finding of gross mass upon imaging was mandatory. Patients with other malignancies were excluded. All patients were identified from local databases and a retrospective chart review was conducted. The presence of a benign dominant stricture was obtained from endoscopic retrograde cholangiography (ERC) findings for patients treated at the University Hospital Heidelberg. ERC had to be performed within one week of CEA to be included. All patients had provided written informed consent and the study was previously approved by local ethics committees in both Heidelberg and Oslo and was conducted in accordance with the Declaration of Helsinki.

Patients who were treated at the University Hospital Heidelberg were included as the primary study cohort. Patients with PSC treated at the Oslo University Hospital were included as an independent control cohort. Genotyping for *FUT2* and *FUT3* was performed and patients were grouped based on these genotypes as described below.

We evaluated the first available CEA values in patients who had no malignancies; in patients with biliary malignancies, we evaluated CEA at the time of primary diagnosis of cancer. A chart review was performed to obtain CEA values determined during routine clinical follow-up. Because distinct database models were used, for some of the patients in the control cohort CEA values were only available rounded to whole numbers. Thus, all CEA values in the control cohort were rounded to whole numbers and were analysed after being grouped in quartiles. Basic demographic and PSC-related health characteristics were also obtained.

Patients: study and control cohorts

Of the 302 patients identified and screened at the University Hospital Heidelberg a total of 226 PSC patients were included in the final study cohort. As shown in Figure 1, 48 of the screened patients were excluded before genotyping because of extrabiliary malignancy ($n = 26$) or because no CEA value was available to meet the inclusion criteria ($n = 22$). After genotyping, 28 more patients were excluded either because the genotype information was incomplete ($n = 24$) or because the *FUT* genotype was inconclusive with regard to the grouping algorithm ($n = 4$). Among the included patients there were 19 (6.3%)

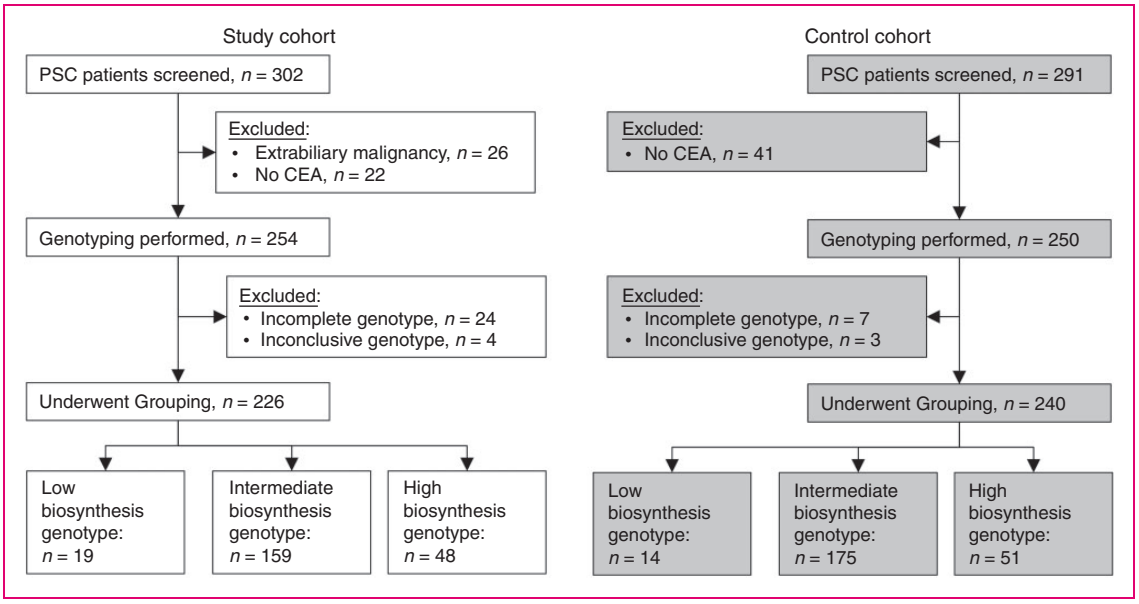

Figure 1. Flowchart showing the recruitment of study patients in both cohorts; information on patients who were excluded from the study is also included.

patients with biliary malignancies ($n = 15$ with CCA and $n = 4$ with GBCA). Nineteen patients (8.4%) were assigned to the low CA19-9 biosynthesis genotype group, 159 (70.4%) to the intermediate group, and 48 (21.2%) to the high biosynthesis genotype group. An ERC was available for 102 (49.3%) patients and in 35 (34.3%) of those patients a dominant stricture was diagnosed in ERC.

In the case of the patients who were treated at the Oslo University Hospital, of the initially 291 screened patients, we excluded a total of 51 (Figure 1). Among the 240 finally included patients, biliary malignancy was detected in 28 patients (11.7%). Fourteen patients (5.8%) belonged to the low CA19-9 biosynthesis genotype group, 175 (72.9%) to the intermediate group, and 51 (21.3%) to the high biosynthesis genotype group (Figure 1).

Basic characteristics of the patients in the two cohorts are compared in Table 1, and data on genotyping results for *FUT2* and *FUT3* are presented in Supplementary Table 1. No statistically significant differences were observed between the cohorts.

Table 1. Patient characteristics. The basic demographic and health characteristics of the study patients were no different between the study cohort and the control cohort

	Study cohort	Control cohort	*P*
Patients, *n* (%)	226	240	
Age, mean (SD), y	38.4 (+/−12.3)	39.5 (+/−13.1)	.427
Male, *n* (%)	160 (70.8%)	176 (73.3%)	.542
IBD, *n* (%)	161 (71.2%)	173 (72.1%)	.840
Malignancy	19 (8.4%)	28 (11.7%)	.243
CCA	15 (78.9%)	27 (96.4%)	
GBCA	4 (21.1%)	1 (3.6)	
CA19-9 biosynthesis group			.550
Low	19 (8.4%)	14 (5.8%)	
Intermediate	159 (70.4%)	175 (72.9%)	
high	48 (21.2%)	51 (21.3%)	

FUT2 and FUT3 genotyping

Genotyping was performed for the *rs601338* (G428A) allelic variant of *FUT2* and for the allelic variants *rs778986* (C314T), *rs812936* (T202C), *rs3894326* (T1067A), and *rs28362459* (T59G) of *FUT3*. For patients in the study cohort that were previously already enrolled in our trial on CA19-9 and for patients in the control cohort, genotyping was performed as previously described.[7] In patients not included in prior analysis the following methods for genotyping were applied at the central laboratory of the Heidelberg University Hospital. Genomic DNA was extracted from whole blood samples using the QIAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany). DNA was analysed on a Light Cycler 2.0 (Roche, Mannheim, Germany) using LightSNiP assays from TibMolBiol (Berlin, Germany) for *rs601338*, *rs812936*, *rs778986* and *rs28362459* according to the manufacturer's instructions. *rs3894326* analysis was performed by Sanger sequencing on an ABI PRISM 310 Genetic Analyser (Applied Biosystems, Darmstadt, Germany), forward

and reverse primer sequences were 5′ACCTG AGCTACTTTCGCTGG3′ and 5′CAAAGGACTC CAGCAGGTGA3′, respectively. In individual cases 5′GCCCCAGGCAGATGAGG3′ was used as an alternative reverse primer.

Patient classification based on FUT *genotypes*

Patients with complete genotype data were grouped into three groups representing patients with genetically determined low (Group A), intermediate (Group B), and high (Group C) CA19-9 biosynthesis activity as described previously.[7] To summarize, grouping was done as following: the patients' enzyme activity of FUT2 and 3 was estimated based on their individual *FUT2* and *FUT3* genotype. Patients with an expected loss of FUT3 enzyme activity were attributed to the low biosynthesis group without consideration of *FUT2* genotype. Only for patients with an expected normal or partly reduced enzyme activity of FUT3 the individual *FUT2* status was taken into account and in case of an expected loss of FUT2 activity the patient was attributed to the high biosynthesis group. If there was expected normal or partly reduced activity for FUT2 and 3 the patient was allocated to the intermediate biosynthesis genotype group. The *rs3745635* (G508A) variant of *FUT3* was not considered for the grouping because its expected occurrence in the study population was low (Supplementary Figure 1).

Statistics

CEA measurement results are presented as medians together with the interquartile range (IQR). The Kruskal–Wallis test and Mann–Whitney *U*-test were used to compare CEA values. To compare the two cohorts, Mann–Whitney *U*-test and chi square test were used as appropriate. The area under the curve (AUC) of a receiver operating characteristic (ROC) was determined. Youden's index was used to determine optimal cut-off values. Sensitivity, specificity, positive and negative predictive values (PPVs and NPVs), and diagnostic accuracy were calculated from a contingency table. Statistical analyses were performed using IBM SPSS version 21. Graphs were generated using GraphPad Prism version 5. Statistical significance was defined as $P < 0.05$

Results

Influence of FUT2 *and* FUT3 *SNPs on CEA levels in cancer-free patients*

The overall median CEA in cancer-free patients in the study cohort was 1.4 ng/mL (IQR: 0.9–2.1). There was no difference between cancer-free patients with (median: 1.5 ng/mL) or without (median: 1.5 ng/mL) benign dominant stricture at time of CEA ($P = 0.514$). The *FUT2* SNP G428A was significantly associated with CEA levels in these patients ($P < 0.001$). Median CEA values according to genotype were 1.1 ng/mL (0.7–1.6) for wild-type (GG), 1.4 ng/mL (0.9–1.9) for heterozygous mutated (GA), and 2.1 ng/mL (1.4–2.9) for homozygous mutated (AA) (Figure 2(a)). No significant associations were observed for the investigated

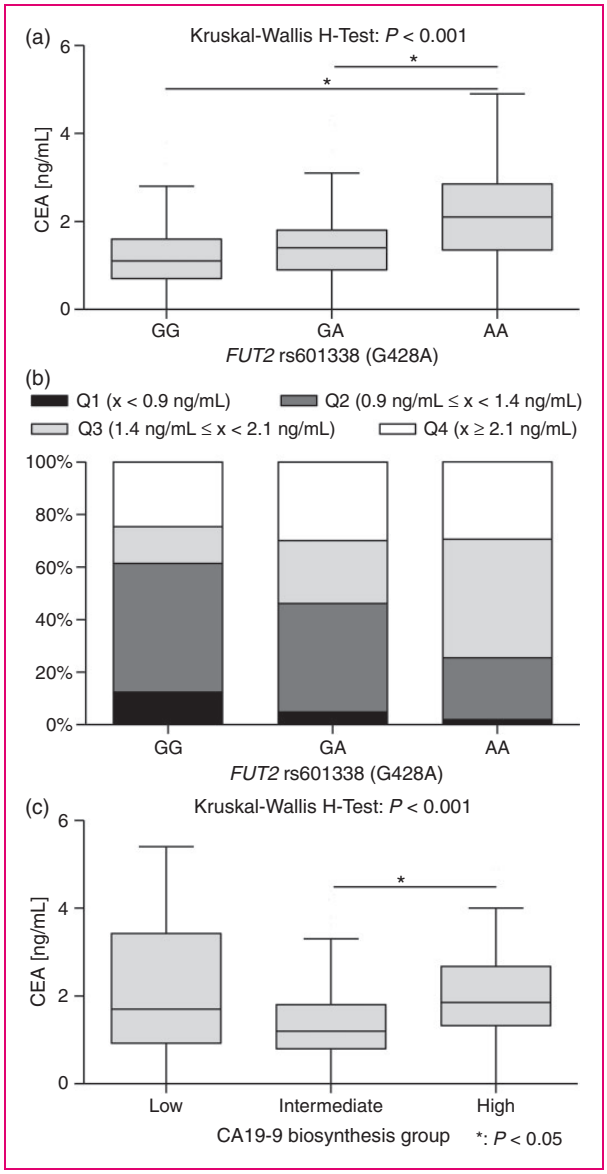

Figure 2. Influence of the *FUT2* variant *rs601338* (G428A) on serum CEA levels in cancer-free patients. Differences in CEA levels in the study cohort (a) and in the control cohort (b) were statistically significant. The CA19-9 biosynthesis group also exerted a statistically significant effect on serum CEA levels in cancer-free PSC patients.

FUT3 SNPs (C314T: $P=0.062$; T202C: $P=0.054$; T1067A: $P=0.202$; T59G: $P=0.449$). Further investigation of the trend for an association with C314T and T202C revealed the highest serum CEA values in patients with the homozygous mutant and the lowest in patients with the heterozygous mutant (C314T, $P=0.066$; T202C, $P=0.045$); patients with the wild-type form showed an intermediate CEA level (Supplementary Figure 2).

Influence of FUT2 *on CEA levels in patients not able to synthesize CA19-9*

Analysis of the G428A variant of *FUT2* in the subgroup of patients incapable of synthesizing CA19-9, namely those in the low biosynthesis genotype group, again showed a significant difference depending on the G428A genotype ($P=0.046$). The resulting median CEA levels were 1.3 ng/mL (0.5–1.7) in patients with the wild-type (GG) *FUT2* SNP, 1.8 ng/mL (0.6–2.3) in patients with the heterozygous mutant (GA), and 4.4 ng/mL (2.7–5.0) in patients with the homozygous mutant (AA). The influence of the homozygous mutation of the *FUT2* SNP was considerably more pronounced in this subgroup of patients than in patients who were capable of synthesizing CA19-9: Patients with a homozygous mutation who were unable to synthesize CA19-9 had a significantly higher CEA level than those capable of CA19-9 biosynthesis (median: 1.9 ng/mL; IQR: 1.3–2.7; $P=0.046$) (Figure 3).

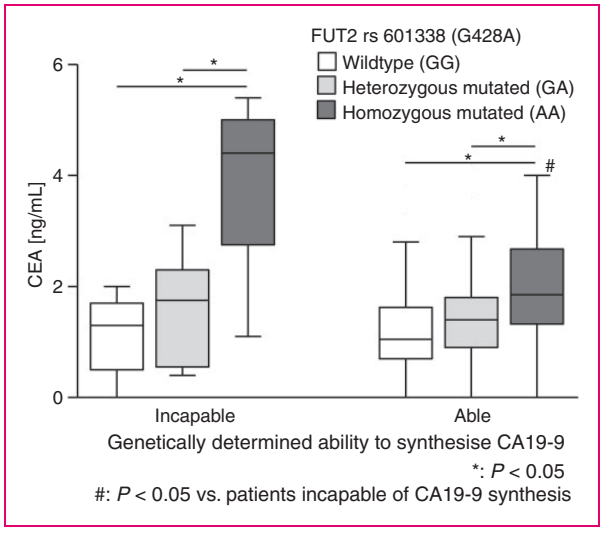

Figure 3. The influence of the *rs601338* variant of *FUT2* on CEA levels was more pronounced in patients who were genetically incapable of synthesizing CA19-9 (i.e. patients in the low CA19-9 biosynthesis group) when compared with the effect in patients who could synthesize CA19-9.

FUT-*dependent CEA values in patients with biliary malignancy*

The serum CEA level of the 19 patients with biliary malignancy in the study cohort was 2.0 ng/mL (IQR: 1.4–3.8, $P=0.014$, compared to cancer-free patients). There was no difference in CEA serum levels between patients with CCA compared to those with GBCA. Median serum CEA levels sorted according to the G428A *FUT2* SNP were 2.3 ng/mL (GG), 1.7 ng/mL (GA), and 80.5 ng/mL (AA), but the differences were not statistically significant ($P=0.219$). There was no significant influence of any of the four *FUT3* SNPs on CEA levels in patients with biliary malignancy.

ROC analysis for determining the optimal cut-off values for detection of biliary malignancy

The AUC, or the detection of biliary malignancy based on ROC analysis, of all patients in the study cohort was 0.671 (95% CI: 0.533 – 0.809, $P=0.014$). The optimal cut-off was 3.2 ng/mL; this resulted in a sensitivity of 36.8% and a specificity of 90.8%. The PPV and NPV values were 26.9% and 94.0%, respectively. The diagnostic accuracy was 86.3%. ROC analysis revealed similar results when only including those cancer-free patients with a benign dominant stricture (AUC: 0.677, 95% CI: 0.524–0.830, $P=0.033$).

ROC analysis performed for the three variants of *FUT2* G428A showed an increased AUC in the case of wild-type patients (AUC: 0.731, 95% CI: 0.506–0.955, $P=0.036$;) and in the case of patients with a homozygous mutation (AUC: 0.816, 95% CI: 0.502–1.000, $P=0.037$). By comparison, the use of CEA resulted in a diminished ability to discriminate between cancer and no cancer in patients with a heterozygous mutation (AUC: 0.610, 95% CI: 0.409–0.810, $P=0.333$). Accordingly, determination of the optimal cut-off yielded different CEA values for the patients in the three groups, as summarized in Table 2.

Influence of the FUT2 *genotype and detection of biliary malignancy in the control cohort*

As noted in the methods section, rounded values were grouped into quartiles to minimize the possible influence of rounding. These quartiles (Q1–Q4) were defined based on the data obtained from the study cohort: $Q1 < 0.9$ ng/mL; 0.9 ng/mL $\leq Q2 < 1.4$ ng/mL; 1.4 ng/mL $\leq Q3 < 2.1$ ng/mL; and $Q4 \geq 2.1$ ng/mL. Figure 2(b) presents the distribution of the quartiles according to the G428A SNP: The data reveal an overrepresentation of the lower quartiles in wild-type patients and an underrepresentation in patients with the homozygous mutation, whereas the contrary is

Table 2. CEA cut-off values. Comparison of distinct CEA cut-off values for the entire study cohort and separated for the three variants of *FUT2 rs601338* (G428A)

	CEA	Sensitivity	Specificity	PPV	NPV	Accuracy
Study cohort	3.2 ng/mL	36.8%	90.8%	26.9%	94.0%	86.3%
Wild-type (GG)	2.2 ng/mL	62.5%	87.3%	41.7%	94.1%	84.1%
Heterozygous (GA)	1.5 ng/mL	71.4%	62.1%	9.4%	96.5%	54.5%
Homozygous (AA)	26.2 ng/mL	75.0%	100.0%	100.0%	98.0%	98.1%

observed in the case of the results of the upper quartiles; in patients with the heterozygous mutation, intermediate results are observed. These results were statistically significant (chi square: 20.670; $P = 0.002$).

We also analysed the rounded CEA values in the control cohort; a significant difference was detected based on the *FUT2* genotype in cancer-free patients ($P = 0.007$). ROC analysis performed using the rounded CEA values revealed an AUC of 0.661 ($P = 0.006$; 95% KI: 0.549–0.774) for discrimination between presence or absence of cancer. In the control cohort, the use of the rounded values also revealed an influence of the *FUT2* variant G428A: AUC was 0.767 ($P = 0.022$; 95% KI: 0.595–0.939) in the wild-type group, 0.590 ($P = 0.234$, 95% KI: 0.438–0.743) in the heterozygous mutant group, and 0.846 ($P = 0.022$, 95% KI: 0.621–1.000) in the homozygous mutant group.

Discussion

We investigated the use of CEA as screening parameter for biliary malignancy in PSC patients. We were able to show that *FUT2* genotype influences CEA serum levels and their interpretation. It was possible to identify patients, in which CEA has a high discriminatory ability and to distinguish them from those, in which there was no discriminatory ability. Additionally, CEA especially seems to be an interesting screening parameter in patients that are incapable of synthesizing CA19-9.

The results of this study support the conclusion that CEA can be used as screening parameter for CCA and GBCA in PSC patients. However, its diagnostic value, as assessed using ROC analysis, is not as strong as it has previously been published for CA19-9,[7] but similar to those of a previous study on CEA in PSC patients, which yielded an AUC of 0.683.[14] Sensitivity and PPV were low for the calculated cut-off of 3.2 ng/mL, whereas specificity and NPV were considerably higher. This could make CEA an interesting parameter for a confirmatory test after screening with a high sensitivity test. Overall, our results are in line with the few previous studies conducted on CEA. In agreement with our results, Ramage et al.[8] reported that sensitivity was low (53.3%) and specificity was high (86.3%) at a cut-off of 5 ng/mL. Another large study on the use of CEA in PSC, conducted by Siqueira and coworkers,[15] recommended a CEA cut-off of 5.2 ng/mL and reported a sensitivity of 62.5% and a specificity of 78.4%. Low sensitivity (33%) and high specificity (85%) were also obtained for CEA at a cut-off of 5 ng/mL by Björnsson and colleagues.[16] Most interestingly however, there seems to be no influence of benign dominant strictures on CEA serum levels or its diagnostic performance in cancer detection.

We investigated the influence of *FUT2* and *FUT3* genotypes on serum CEA levels and on its diagnostic performance in PSC patients. These genotypes were recently shown to strongly affect CA19-9 levels in PSC patients and influence the diagnostic performance of CA19-9.[7] Although two recent GWAS have reported an association between *FUT2* and CEA,[9,17] in this study we have shown for the first time that this association is clinically relevant and that it positively influences the diagnostic performance of CEA. Our results showed that CEA levels increased with the number of A alleles in the case of the *rs601338* variant of *FUT2*. While the overall ability to discriminate between tumour and tumour-free patients was only moderate, we showed great improvement when analysed separately for patients with the wild-type and homozygous mutant forms of this SNP and we were also able to define individual cut-off values. The dependence of CEA levels on the *FUT2* genotype was confirmed in an independent control cohort. To allow a clinical implementation of this approach, we as well calculated *FUT2* depending cut-off values with a sensitivity of 95% and 99%, respectively (Supplementary Table 2). Overall, this study represents a necessary step to be taken in the progression from the detection of an association between CEA levels and the *FUT* genotype in the two GWAS to the development of personalized medicine for use in daily clinical practice. This approach could also be of considerable interest in the case of other more common gastrointestinal malignancies. It should now be evaluated whether *FUT2* dependent CEA serum levels not only improve accuracy in tumour screening, but could also be of help for detection of cancer recurrence after initial therapy.

Overall, *FUT2* gene becomes increasingly interesting in the setting of PSC.[18] It was shown to be associated with PSC in a large GWAS,[19] and was identified as a risk factor for dominant stenosis and cholangitis in these patients.[20] It further is of interest because it has an influence on serum levels of CA19-9 and CEA, which are both used for cancer screening in PSC.

In contrast to *FUT2*, the four *FUT3* variants did not exert statistically significant effects on serum CEA levels; however, we noted one intriguing point: in the subset of patients who were unable to express CA19-9, as determined by *FUT3* genotype, the influence of *FUT2* appeared to be even more pronounced. However, we could not analyse this further because number of patients with biliary malignancy became too low. Nevertheless, because these patients cannot synthesize CA19-9, distinct screening parameters, such as CEA levels, must be used. Therefore, the influence of *FUT2* in this group of patients warrants further investigation. The influence of *FUT2* and *FUT3* on CA19-9 and CEA seems to be genetically determined, thus we believe that our findings will hold true for other gastrointestinal cancers as well, as has already been shown for CA19-9[10] and as it is suggested for CEA.[17]

Despite these very promising, novel findings, we note that there are some minor limitations within our study. In the control cohort CEA values were only available as rounded values. We were thus not able to actually validate results with regard to cut-off values in this cohort. Nevertheless, we were able to prove the influence of *FUT2* on CEA *per se*. Regardless of its retrospective design, our study still profits from inclusion of two independent, well-characterized and large cohorts of PSC patients. It is the largest study on CEA in this setting so far, and we were also able to include a considerable number of patients with biliary malignancies. However, further studies on other tumour entities are now needed to confirm the results.

Overall, our findings support the results from two recent GWAS on the association between *FUT2* and CEA, but further show that *FUT2* genotyping helps improve the discriminatory ability of CEA. The latter observation could also be of interest in the case of gastrointestinal malignancies that are more common than CCA, such as colorectal cancer, and should, therefore, be further investigated. In combination with the *FUT2* genotype, the CEA level is an interesting screening parameter and could be particularly valuable in the case of patients who cannot express CA19-9 and warrants further investigations. Meanwhile, we recommend screening PSC patients by using CA19-9 and CEA.

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Conflict of interest statement

None declared.

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